REMARKS

The claims have been amended to address the objections under 35 U.S.C. § 112, paragraph 2, and to further clarify the invention. New claims 72-75 are supported, for example, by claim 16, Examples 7 and 8, and throughout the specification. New claims 76-77 are supported by current claims 7 and 25.

Claims 1 and 18, the only independent claims, have been amended to clarify that the nanoparticles are liquid when bound to the target, and indeed when the acoustic image is taken. This limitation clearly distinguishes the work of Unger who employs either solids or gas bubbles as contrast agents for acoustic imaging. Also in these claims, the step of administering the nanoparticles in a non-gaseous emulsion has been removed as an active step. This permits the foregoing clarification regarding the physical state of the nanoparticles when bound to the target and also prevents any issue regarding avoiding infringement should the step of administering and the step of changing the temperature and imaging be performed by different entities, unlikely as this appears.

Claim 19 has been amended to clarify that it includes further steps other than those set forth in claim 1. Claims 13 and 31 have been amended simply for clarification; shortwave, microwave and magnetic radiation are all forms of electromagnetic energy. Sufficient temperature change must be effected so as to enhance acoustic reflectivity. A precise measure of the temperature rise is provided in claims 17 and 35; however, the effect is continuous, so as long as a change in reflectivity is detectable, it is included in the scope of the claims.

Claims 15-16 and 33-34 have been canceled as inconsistent with the claim from which they depend. No new matter has been added and entry of the amendment is respectfully requested.

The Invention

As discussed at the interview, the invention resides in the surprising finding that by raising the temperature of nanoparticles bound to a target, the image of the target can be improved by virtue of raising the temperature of a liquid nanoparticulate contrast agent. The temperature rise does not convert the liquid nanoparticles to gas bubbles as described by Unger; rather, as demonstrated in the specification, raising the temperature enhances the image. Conversely, the image can be changed by lowering the temperature of the nanoparticles. The nanoparticles are administered in an emulsion which is itself nongaseous. The emulsion can further include a biologically active agent. Respectfully, applicants do not believe that any of the cited documents suggest the invention as claimed.

The Rejection Under 35 U.S.C. § 112, Second Paragraph

It is believed that the amendments to the claims are responsive to this rejection. The term "ultrasound target" does not appear in the claims; however, "target for ultrasound imaging" does. Target for ultrasound imaging, of course, refers to a material or, if *in vivo*, a tissue or organ for which an ultrasound image is desired. The nature of such targets is described extensively in the specification. For example, the target may be as simple as a nitrocellulose membrane (Figure 2) or human plasma clots (Figure 5) and on page 12, line 25, it is clarified that the target may be an *in vivo* or *in vitro* target and preferably a biological material. As stated, it may be a surface to which the acoustic contrast substance binds or a three-dimensional structure. As the Office may be aware, typical ultrasound targets in a medical context would include various organs such as liver or lungs. Ultrasound imaging to follow development of a fetus is a widely understood application.

As to what is bound to the target, the claims have clarified that it is the nanoparticles which are bound. The term "the bound emulsion" no longer appears in the claims.

The Rejection Under 35 U.S.C. § 102(b) Over Milbrath (U.S. 5,401,634)

Reconsideration of this rejection is requested. The passages referred to in Milbrath that putatively involve enhancing the temperature involve spectroscopic measurements. There is no evidence that spectroscopic measurements would change the temperature of any bound nanoparticles in a manner sufficient to produce a measurable change in acoustic reflectivity of the target as required by the claims.

In addition, clearly claims 72-75 are free of Milbrath as they require *in vivo* methods.

As discussed at the interview, simply measuring the absorbance of light by a material does not raise the temperature of that material. Visible light, such as that described in the cited portions of Milbrath, is of appropriate energy to effect electronic excitation, not to enhance the vibration or rotational states of the molecules. Appropriate electromagnetic radiation to result in a temperature rise would be in the microwave range, well below the wavelengths employed by Milbrath. Thus, there is no inherent anticipation by Milbrath; certainly there is nothing in Milbrath which states that the temperature of the nanoparticles described by Milbrath is raised.

Applicants further point out that claim 18 and its dependent claims cannot be anticipated by Milbrath because, as amended, they require obtaining an acoustic image of the target. This step is not performed by Milbrath. Therefore, currently pending claims 18, 21, 25-26, 31, 35, and 70-71 and 77 (as well as new claims 72-75) are free of this rejection. Claim 19 is not anticipated because it requires measuring reflectivity.

In addition, claims 3, 7, 14 (whose limitations are now included in claim 13) were never included in this rejection.

The Rejection Under 35 U.S.C. § 103 Over the Combination of Trevino (U.S. 5,733,526), Allen (U.S. 5,527,528) and Unger (U.S. 6,123,923)

This rejection was applied to all claims. Respectfully, applicants believe there is no motivation to combine the documents cited, and even if combined, it does not appear that the combination suggests the invention. Applicants are unable to find anything in any of these documents which suggests that the reflectivity of an ultrasound image could be changed by changing the temperature. The only document that is said to discuss ultrasound imaging at all, Unger, makes no mention of changing the temperature in order to change the reflectivity. Also, the referred-to sections, other than column 121, lines 19-25, do not seem to concern scanning by ultrasound. As discussed at the interview, these sections were apparently cited for alternative limitations, not of concern with respect to the independent claims.

Turning first to the disclosure of Trevino, the emulsions that are the focus of this document apparently contain fluorocarbons as a continuous phase with oil particles interspersed or are multiple emulsions. There is no emulsion in Trevino that is even similar to that of claims 7, 25, 77 and 78 - *i.e.*, the nanoparticles themselves are not fluorocarbons encapsulated with a lipid surfactant. As noted, for example, in column 1, lines 6-11, the Trevino emulsions are hydrocarbon oil-in-fluorochemical dispersions or hydrocarbon oil-in-fluorochemical-in water emulsions. They are not the type of emulsions administered according to the present claims which result in liquid nanoparticles bound to a target. The emulsions do not contain, as best applicants can tell, liquid nanoparticles comprising fluorocarbons at all.

In addition, the allusion to therapeutic and diagnostic use noted by the Office in column 13, line 60-column 14, line 15 makes no mention of ultrasound imaging, nor is ultrasound imaging mentioned anywhere in the document.

Further, there is no mention of particle size that applicants have found in the generic description of the microemulsions - the term "microemulsion" would imply particle sizes in the micron range, which is an order of magnitude greater than nanoparticles. The Office points to Example 4 where small droplets of $\leq$100 nm are formed; this is also the case in Example 3; however, Example 6 results in particles of 10 μm. Thus, it appears that the only disclosure of nanoparticulate preparations in Trevino is in Examples 3 and 4. Example 3 does not describe nanoparticles comprising fluorocarbon; rather, the fluorocarbon, as noted, is the continuous phase and the particles are particles of oil. They are maintained in the continuous phase by a micellular coating which does not form part of any nanoparticles. Similarly, in Example 4, methyl salicylate dissolved in oil droplets is contained in a continuous fluorocarbon phase. Thus, Trevino does not describe even non-targeted liquid nanoparticles comprising fluorocarbons.

The Office cites Allen to show that attaching a targeting agent such as an antibody to the surface of "lipid vesicles" is well known. What Allen does not teach, however, is that attachment of ligands such as an antibody to *nanoparticles comprising fluorocarbon* is well known. Allen merely teaches that attachment of antibodies to liposomes, an entirely different type of delivery vehicle. No agent is suggested, at least in the Allen document, for use in ultrasound. Allen is silent on any attachment of targeting ligands to liquid nanoparticles comprising fluorocarbons intended for ultrasound imaging.

As recognized by the Office, Unger requires either gaseous forms of fluorocarbons or solid matrices that contain gases. Unger discloses ultrasound imaging using these particles, which are, applicants emphasize, not liquid nanoparticles, but fails to disclose any suggestion that raising the temperature is desirable to enhance the image. Indeed, Unger teaches away from

the present invention by emphasizing that any temperature rise is undesirable. As noted in column 93, at line 55,

> vesicles will rupture when exposed to non-peak resonant frequency ultrasound in combination with a higher intensity (wattage) and duration (time). This higher energy, however, results in greatly increased heating which may not be desirable. By adjusting the frequency of the energy to match the peak resonance frequency, the efficiency of rupture and release of the photoactive agent is improved, appreciable tissue heating does not generally occur (frequently no increase in temperature above about 2°C) and less overall energy is required. Thus, application of ultrasound at the peak resonant frequency, while not required, is preferred.

Unger uses this higher energy in order to rupture the vesicles to deliver the photoactive agent. In order to obtain an ultrasound image, such higher energy is not employed. As the claims require that the nanoparticles be liquid, and thus not be ruptured, this higher energy, which would have caused the temperature increase, is not suggested with respect to the claimed method by the disclosure of Unger.

In summary, the combination of Trevino, Allen, and Unger, even if made, does not suggest the invention. First, there is no disclosure in any of the three documents of liquid nanoparticles comprising fluorocarbons. Second, there is no suggestion in any of the three documents to target *liquid nanoparticles* (Unger suggests targeting gaseous particles - *e.g.*, column 43, line 15 to column 60, line 10). Third, there is no suggestion in any document that liquid nanoparticles be imaged in an ultrasound image at an elevated temperature in order to enhance the quality of the image. Any temperature rise during imaging is taught away from by Unger. Thus, even combining these documents does not result in the invention, which requires liquid nanoparticles bound to a target and increasing the temperature in order to enhance ultrasound image.

It appears that the Office recognizes that the essential element of the invention - enhancing the image by increasing the temperature does not result from this combination. As stated by the Office, the claims are rejected because it would have been obvious to link a targeting ligand to the nanoparticles of Trevino as taught by Allen and Unger and employ an external source of energy as taught by Unger to detect the location of the administered particles at the site of interest. There is no mention in this summary of the rejection of any suggestion to increase the temperature of the nanoparticles in order to enhance the quality of the image.

Further, the motivation suggested does not appear to follow the recognized rationales for motivation as set forth by the Federal Circuit in *In re Rouffet*, 47 USPQ2d 1453 (Fed. Cir. 1998). According to the decision in this case, there are three recognized reasons which would support combination. One is a suggestion in the documents themselves; the Office has pointed to no suggestion in any of Trevino, Allen or Unger that it be combined with disclosures such as those set forth in the remaining documents. The second possible rationale is the nature of the problem to be solved. The problem to be solved in the present invention is to enhance the quality of imaging. Neither Trevino nor Allen even concern imaging. The problem solved by Trevino and Allen is evidently to deliver bioactive agents. The problem addressed by Unger is to improve imaging, but the solution is entirely different from that of the present invention - Unger adds a photoactive agent to the ultrasonic imaging. The third possible rationale is the notorious character of at least one of the documents, a criterion that does not fit here.

In summary, there is no legally sufficient motivation to combine Trevino, Allen and Unger. Even when combined, these documents do not suggest the invention as claimed. Respectfully, applicants request that the rejection of the pending claims over this combination be withdrawn.

Issues Raised at the Interview

Concern was expressed at the interview that "raising the temperature...to produce a measurable enhancement in acoustic reflectivity of the target" was not sufficiently precise. The claims are directed, however, to a method for enhancing acoustic reflectivity of a target or to a method for obtaining an image resulting from enhanced acoustic reflectivity of a target. As long as the temperature is raised sufficiently to achieve this result, the temperature increase is sufficient. The metes and bounds of the claim are thus clear - if the acoustic reflectivity is enhanced so that enhancement is detectable, the temperature rise is sufficient. As the reflectivity apparently rises in a continuous manner with rise in temperature, any specified temperature range would, by necessity, simply be arbitrary.

The Office further objects that there may be an inherent anticipation of the claims due to an inadvertent increase in temperature when liquid nanoparticles comprising fluorocarbons are imaged. However, the ultrasound frequencies used to create images are not the ultrasound frequencies used to enhance temperature - those used to enhance temperature are of longer wavelength and higher intensity. The Office has pointed to no document wherein the temperature of liquid fluorocarbon-based nanoparticles is inherently increased. The only document asserted in this regard is Milbrath which involves spectrophotometric measurements, a procedure which does not result inherently in any increase in temperature.

Finally, the Doppler effect in ultrasound imaging was mentioned. This is not relevant to the present claims as Doppler shifts are used in imaging moving particles not in obtaining an image using targeted nanoparticles.

CONCLUSION

The claims have been amended to clarify the nature of the invention. It is believed that the amendments to the claims are responsive to the rejections under 35 U.S.C. § 112, paragraph 2. The claims are not anticipated by Milbrath because Milbrath does not describe changing the temperature of fluorocarbon nanoparticles at all, much less in order to enhance an ultrasound image. The claims are not rendered obvious by the combination of Trevino, Allen and Unger because there is no motivation for the combination and because once combined, there is still no suggestion to enhance an image by raising the temperature of bound liquid nanoparticles. Thus, applicants respectfully request that the pending claims, claims 1, 3, 7-8, 13, 17-19, 21, 25-26, 31, 35 and 68-77 be passed to issue.

Applicants want to express their appreciation again for the thoughtful discussion provided at the interview. Should any questions remain regarding the issues addressed above, a telephone call to the undersigned is respectfully requested. It may be possible to resolve any outstanding issues over the phone.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicants petition for any required relief including extensions of time and authorize the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket No. 532512000500.

Respectfully submitted,

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EXHIBIT A. - VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

1. (Twice amended) A method for [changing] enhancing acoustic reflectivity of [an] a target for ultrasound [target] imaging, the method comprising [(1) administering to the target, a nongaseous emulsion comprising nano-particles that comprise a liquid fluorocarbon which binds to the target and produces a change in acoustic reflectivity with a change in temperature and (2) changing] raising the temperature of liquid nanoparticles [the] bound to said target [emulsion] to produce a measurable [change] enhancement in acoustic reflectivity of the target, wherein said nanoparticles comprise at least one fluorocarbon, said nanoparticles having been administered to said target in a non-gaseous emulsion.

7. (Twice amended) The method according to claim 1 wherein the nanoparticles [are] comprise at least one liquid fluorocarbon encapsulated with [a] at least one lipid surfactant which comprises [a] at least one ligand that binds to said target.

13. (Twice amended) The method according to claim 1 wherein [changing] raising the temperature comprises providing the target with ultrasound or electromagnetic energy or a combination thereof, sufficient to raise [energizing] the [bound emulsion to increase] temperature of [the bound emulsion and] said nanoparticles, so as to enhance acoustic reflectivity [of the target].

17. (Twice amended) The method according to claim 1 wherein changing the temperature comprises changing the temperature of the bound [emulsion] nanoparticles by at least 5°C.

18. (Thrice amended) A method for [measuring] obtaining an image resulting from enhanced acoustic reflectivity of [an] a target for ultrasound [target] imaging, the method comprising [(1) administering to the target, a nongaseous emulsion comprising nanoparticles that comprise a liquid fluorocarbon which binds to the target and produces a change in acoustic reflectivity with a change in temperature and (2)] changing the temperature of the liquid

nanoparticles bound [emulsion] to said target to produce a measurable [change in] enhancement of acoustic reflectivity of the target, and [(3) detecting change in acoustic reflectivity of the target] obtaining an ultrasound image of said target, bound to said liquid nanoparticles, wherein said nanoparticles comprise at least one fluorocarbon, said nanoparticles having been administered to said target in a non-gaseous emulsion.

19. (Twice amended) The method [according to] of claim [18 wherein detecting] 1 which further comprises (a) measuring reflectivity prior to [changing] raising the temperature of the bound [emulsion] nanoparticles; (b) measuring reflectivity after [changing] raising the temperature of the bound [emulsion] nanoparticles; and (c) determining the change in reflectivity after [changing] raising the temperature of the bound [emulsion] nanoparticles compared to reflectivity prior to [changing] raising the temperature of the bound [emulsion] nanoparticles.

25. (Twice amended) The method according to claim 18 wherein the nanoparticles [are] comprise at least one perfluorocarbon encapsulated with [a] at least one lipid surfactant which comprises [a] at least one ligand that binds to said target.

31. (Twice amended) The method according to claim 18 wherein changing the temperature comprises [energizing the bound emulsion to increase temperature of the bound emulsion and enhance acoustic reflectivity of the target] providing the target with ultrasound or electromagnetic energy or a combination thereof, sufficient to raise the temperature of said nanoparticles, so as to enhance acoustic reflectivity.

35. (Twice amended) The method according to claim 18 wherein [changing] raising the temperature comprises [changing] raising the temperature of the bound [emulsion] nanoparticles by at least 5°C.

69. (Amended) The method according to claim 68 wherein the [ligand] polypeptide is at least a portion of an antibody.

71. (Amended) The method according to claim 70 wherein the [ligand] polypeptide is at least a portion of an antibody.